GC–MS, FTIR FINGERPRINT OF METHANOL AND ETHYL ACETATE ROOT BARK EXTRACT OF ZANTHOXYLUM TESSMANNI (ENGL.) AYAFOR [RUTACEAE]

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ABSTRACT
The use of Zanthoxylum tessmanni (Engl.) Ayafor [Family RUTACEAE] stem and root bark have been in existence from time immemorial. Some acclaimed ethnomedicinal uses of extract of different parts of this plant include: anti-microbial, anti-inflammatory, anti-cancer and cholesterol lowering effect. The phytochemical constituents of Z.tessmannii root bark extract were analyzed using UV-Vis spectrophotometry (UV-2500pc Spec), Fourier Transform Infra-Red spectroscopy (FTIR model-8400S spec.) and Gas Chromatography – Mass-Spectrometer (GC-MS) (model-QP 2010 plus Spec). The compound detection employed the NIST ver.2.0-year 2005 library. The biological activity is based on Dr. Dukes Phytochemical and ethnobotanical databases. Peaks corresponding to polyphenols and its congeners were revealed by the UV-Vis spectroscopy. The FTIR spectrum confirms the presence of alcohols, alkanes, alkenes, ethers, esters and carboxylic acid functional groups. The chemical fingerprint of methanol and ethyl acetate root bark extract of Z.tessmannii revealed 16 prominent phytochemicals with varying medicinal activities. However, Stearic acid (C18H36O2) with retention time of 16.091 and peak area of 13.30% and Furo[2,3-b]quinoline-4(9H)-one,7,8-dimethoxy-9-methyl (C19H16NO3) with retention time of 17.820 and peak area of 25.21% were the prevailing compounds. The presence of these phyto-compounds in the plant provides a leading evidence for their use as chewing stick to keep the mouth odour free.

KEYWORD: Anti-inflammatory; Cholesterol; Ethnobotanical; Phytochemicals; Antimicrobial.

INTRODUCTION
Zanthoxylum tessmannii (Engl.) Ayafor [Family RUTACEAE] is a tree that grows up to 33 m high, bearing thorns towards the base. The branches are thorny; they are found flourishing in the high-forest zones of Liberia, Southern Nigeria and Congo.[1] Previous research has shown that Zanthoxylum tessmannii possesses some antiseptic[2] and astringent properties, antioxidant[3], antibacterial[4] and immune-busting activities.[5] Ethno-medical uses include: chewing stick, for brushing the teeth early in the morning by the indigines of Southern Nigeria. The above usage were claimed to make the mouth fresh and odour free, thus providing a good oral hygiene. Hence the present research is aimed at characterizing the pharmacologically active principle present in the Methanol and Ethyl acetate root bark extract using Ultraviolet-Visible spectrophotometry (UV-Vis), Fourier Transform Infrared spectroscopy (FTIR) and Gas-Chromatography-Mass spectrometry (GC-MS) and pointing out the similarities with already established databases.

MATERIALS AND METHODS
Chemicals
Methanol, n-Hexane, de-ionised water and Ethyl acetate were all of analytical grade and purchased from JHD Chemicals, China.

Plant Material and Extraction
The roots and root bark of Z.tessmannii were collected in the South-Eastern Nigeria at the month of August 2017. The samples were identified and authenticated by Dr. Osuala of the Natural product Unit, University of Port Harcourt, Nigeria. A voucher specimen (UPNP 2000/2017) of the sample was deposited in the institutional herbarium for future use.

Preparation of extract
The collected roots of Z.tessmannii were air-dried under shade for 7 days. The dried root bark was ground into coarse powder using a Willey mill (Thomas Willy mills, Swedesboro, NJ, USA). A 1000 g of the pulverised root bark sample was macerated in 2.5 L of n-Hexane for 72 hrs (de-fating) and filtered. The resultant marc was air dried and re-macerated in 2.5 L of Methanol, the above
The resultant sticky extracts were collected and stored in a refrigerator (-10 °C) till use.

Characterisation of Z. Tessmannii Methanolic and Ethyl acetate Extracts

UV-Vis. And FTIR Spectroscopic analysis

UV-Vis spectrophotometric analysis was employed on the Z. Tessmannii extracts using a UV-Vis spectrophotometer (Perkin Elmer, USA Model: Lambda 850) with a slit width of 2 nm, using 10 mm cell at ambient temperature. Extract was centrifuged at 3000 rpm for 15 min and filtered through 0.450µm sythered glass funnel. The sample was diluted to 1:10 with same extracting solvent prior to analysis. The extract was scanned under visible and UV light in the wavelength ranging from 300-800 nm. The identity of the characteristic functional groups in the extract was revealed using Fourier transform infrared (FTIR) spectroscopy. It afforded a whole range of information about the structure of molecules of phytochemical origin present in the extract. A quantum of the Z. tessmannii extract was mixed in dry potassium bromide (KBr). The mixture was carefully mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. The disc so formed was placed in a sample cup of a diffuse reflectance accessory; the IR spectrum was then generated using Bruker, Germany vertex 70 infrared spectrometer. Sample scanning was carried out from 4000 - 400 cm⁻¹. The peak values of the UV-Vis and FTIR were studied.

GC-MS analysis

Presence of active phytochemical constituents of Z. tessmannii plant extract was identified and characterized by the use of Gas Chromatography and Mass Spectrometry (GC-MS Shimadzu QP-2010 Plus) equipped with thermal desorption system (TD-20). Column specification was Rtx-5 of 30 m x 0.25 x 0.25 µm size. The column temperature was maintained at 100°C in a stepwise increment of 280°C at a rate of 5°C/min, maintained for 5 minutes. The running temperature was later increased to 280°C at a rate of 15°C/min maintained for 35 minutes. The mass spectrometer ion source was held at 230°C. Analysis and detection was made in full scan mode from m/z 40 -700. The characterization and identification of the compounds was effected by comparing obtained mass spectra of unknown peaks with those of standard and NIST (National Institute of Standards and Technology) Libraries.

RESULTS AND DISCUSSION

Identification of phyto-compounds containing δ-bonds, π-bonds, lone pair of electron, chromophores and aromatic π-bonds were made through the use of UV-Vis spectroscopy. Quantitatively, the UV-Vis profile of both the methanolic and Ethyl acetate extract of Z.tessmannii was taken at the wavelength of 300 nm-800 nm, this region presents a sharp and clearer baseline. The corresponding peaks and absorbance spectrum are shown in table1. Peaks around 310 nm and 420 nm confirm the presence of organic chromophores within the Z.tessmannii extract. For the Methanol extract, a shoulder between 300 nm and 335 nm depicts a simple phenol, alcohols and possibly polyphenols. Whereas, peak at 335 nm, could still be attributed to carotenoids.

Table 1: UV-Vis peak values of Methanol & Ethyl acetate extract of Z.tessmannii root bark.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Methanol extract</th>
<th>Ethyl Acetate extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absorbance</td>
<td>Wavelength (nm)</td>
</tr>
<tr>
<td>310.00</td>
<td>08.00</td>
<td>310.00</td>
</tr>
<tr>
<td>316.00</td>
<td>08.50</td>
<td>318.00</td>
</tr>
<tr>
<td>326.00</td>
<td>10.00</td>
<td>325.00</td>
</tr>
<tr>
<td>335.00</td>
<td>12.00</td>
<td>356.00</td>
</tr>
</tbody>
</table>
Figure 1: UV-Vis spectra of pure Ethyl acetate extract of Z.tessmannii.

Figure 2: UV-Vis spectra of pure Methanol extract of Z.tessmannii.

**FTIR analysis**

Identification of the functional groups of the active phytochemical compounds was achieved using the FTIR spectrum. This was based on the peak value in the region of infrared radiation. The FTIR spectrum of the *Z.tessmannii* root bark extract in KBr disc is shown in fig.3 and 4 for Methanol and Ethyl acetate respectively.
**Fig 3:** FTIR spectrum of methanol root bark extract of Z. tessmannii.

**Fig 4:** FTIR spectrum of ethyl acetate root bark extract of Z. tessmannii.

**Table 2:** FTIR Peak value and corresponding functional groups of the different – Phytochemical Compounds present in the Methanol Extract of Zanthoxylum tessmannii root bark.

<table>
<thead>
<tr>
<th>Peak Values (Cm⁻¹)</th>
<th>Description</th>
<th>Likely Group Present</th>
<th>Functional Group Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2937.1</td>
<td>Weak, Intermediate</td>
<td>Terminal C-H of alkyl, alkene</td>
<td>C-H</td>
</tr>
<tr>
<td>1449.9</td>
<td>Weak, Intermediate</td>
<td>C-H bending</td>
<td>C-H</td>
</tr>
<tr>
<td>1256.1</td>
<td>Very Weak</td>
<td>C-O stretching in ether, alcohols and esters</td>
<td>C-O</td>
</tr>
<tr>
<td>3258.9</td>
<td>Broad, Strong</td>
<td>O-H of an alcohol or phenol</td>
<td>O-H</td>
</tr>
<tr>
<td>1017.6</td>
<td>Strong</td>
<td>C-O stretching in alcohols, ethers</td>
<td>C-O</td>
</tr>
</tbody>
</table>

**Table 3:** FTIR Peak value and corresponding functional groups of the different – Phyto-Components identified in the Ethyl acetate Extract of Zanthoxylum tessmannii root bark.

<table>
<thead>
<tr>
<th>Peak Values (Cm⁻¹)</th>
<th>Description</th>
<th>Likely Group Present</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3354.8</td>
<td>Broad and Weak</td>
<td>O-H of an alcohols, Carbonyl-OH</td>
<td>O-H</td>
</tr>
<tr>
<td>2926.0</td>
<td>Strong and Sharp</td>
<td>Terminal C-H of an alkyl, alkene</td>
<td>C-H</td>
</tr>
<tr>
<td>2855.1</td>
<td>Sharp, Variable</td>
<td>C-H stretching of alkane, alkene</td>
<td>C-H</td>
</tr>
<tr>
<td>1722.0</td>
<td>Sharp</td>
<td>C=O Stretching of Carbonyl</td>
<td>C=O</td>
</tr>
<tr>
<td>1654.9</td>
<td>Weak</td>
<td>C=C Stretching</td>
<td>C=C</td>
</tr>
</tbody>
</table>

**GC-MS Analysis**

The phytochemical constituents identified by the GC-MS analysis of the methanolic and Ethyl acetate extracts of Z. tessmannii was highlighted along with their molecular formula, retention time and peak area. The chromatogram (Fig.5) of methanolic extract showed
some prominent peaks as Glycerin, \((C_3H_8O_3)\) with retention time of 3.187 and peak area of 4.06%, -Octanol,2-butyl-s \((C_{12}H_{26}O)\) with retention time of 3.458 and peak area of 4.61%, Cyclotetra siloxane, octamethyl- \((C_8H_{24}O_4Si_4)\) with retention time of 5.308 and peak area of 4.70%, 1-Butanol,4-butoxy- \((C_6H_{12}O_3)\) with retention time of 5.549 and peak area of 2.02%, Butanoic acid, 2-hydroxy-2-methyl- \((C_4H_7O_2)\) with retention time of 5.749 and peak area of 3.11%, 2H-Pyran,5,6-dihydro-5-acetoxy-2-[4-(+)]- \((C_4H_5O_3)\) with retention time of 6.093 and peak area of 5.98%, Benzoic acid, 2,5-bis (trimethylsiloxy)- \((C_{10}H_{10}O_2Si_3)\) with retention time of 6.919 and peak area of 2.85%, Dimethyl Phthalate \((C_{10}H_{10}O_2)\) with retention time of 7.852 and peak area of 5.80%, Phthalic acid, cyclobutyl ester \((C_{25}H_{38}O_4)\) with retention time of 13.299 and peak area of 1.50%. Palmitic acid \((C_{10}H_{20}O_2)\) with retention time of 14.257 and peak area of 0.15%. Chromatogram of Ethyl acetate extract revealed some prominent peaks including: 1,2,3-Propanetriol, monoacetate \((C_3H_6O_2)\) with retention time of 4.386 and peak area of 4.92%, Benzoic acid,4-formyl- \((C_6H_2O_2)\) with retention time of 6.715 and peak area of 0.350%, 4-Fluorobenzoic acid,1-cyclopentylethyl ester \((C_8H_7F)\) with retention time of 9.883 and peak area of 1.91%, 1-(+)-Ascorbic acid 2,6-dihexadecanooate \((C_{36}H_{72}O_8)\) with retention time of 14.059 and peak area of 1.48%, 1,7-Dimethyl-4-(1-methyl ethyl) cyclodecane \((C_{13}H_{28}O_2)\) with retention time of 14.283 and peak area of 7.69%, 9-Tetradecenal \((C_{14}H_{26}O)\) with retention time of 15.720 and peak area of 9.53%, Stearic acid \((C_{18}H_{36}O_2)\) with retention time of 16.091 and peak area of 13.30%, Furo[2,3-b]quinolin-4(9H)-one,7,8-dimethoxy-9-methyl \((C_{14}H_{13}NO_4)\) with retention time of 17.820 and peak area of 25.21%.

![Fig 5: Gas chromatogram of the methanol root bark extract of Z.tessmannii.](image)

![Fig 6: Gas chromatogram of the ethyl acetate root bark extract of Z.tessmannii.](image)
SOME STRUCTURES OF THE IDENTIFIED PHYTO-COMPONENTS IN THE METHANOL EXTRACT OF ROOT BARK OF *Zanthoxylum tessmannii* (GC-MS)

(1) 1, 2, 3-Propanetriol (glycerin) (C₃H₈O₃)

(2) 1-Octanol, 2-butyl (C₁₂H₂₆O)

(3) 1, 2, 3-Propanetriol, monoacetate (C₅H₁₀O₄)

(4) 1, 2, 4-Butanetriol (C₄H₁₀O₃)

(5) Cyclotetrasiloxane, Octamethyl (C₈H₂₄O₄Si₄)

(6) 1-Butanol, 4-Butoxy (C₈H₁₈O₂)

(7) 1-Hexene-4-ol, 4-cyclohexyl-3-methyl (C₁₃H₂₅O)

(8) Furan, 2-hexyl (C₁₀H₁₆O)

(9) Dimethyl phthalate (C₁₀H₁₀O₄)

(10) Oleic acid (C₁₈H₃₄O₂)

(11) Stearic acid (C₂₀H₄₀O₂)

STRUCTURES OF THE IDENTIFIED PHYTO-COMPONENTS IN THE ETHYL ACETATE EXTRACTS OF ROOT BARK OF *Zanthoxylum tessmannii* (GC-MS)

(1) Benzoic acid, 4-Formyl

(4) (+)-Ascorbic acid, 2, 6-dihexadecanoate (C₃₉H₆₈O₈)
(2) 3-Hydroxy-4-methoxybenzoic acid (C₈H₆O₄)
(5) Furo [2, 3-b] quinolin-4-(9H)-one, 7, 8-dimethoxy-9-methyl (C₁₄H₁₃NO₄)
(3) 4-Fluorobenzoic acid, 1-cyclopentylethyl ester (C₁₄H₁₇FO₂)

Table 4: Biological Activities of some Active Principles found in the Methanol and ethylacetate extracts of the root bark of Zanthoxylum tessmannii.

<table>
<thead>
<tr>
<th>PYTOCOMPONENTS</th>
<th>NATURE OF COMPOUNDS</th>
<th>BIOLOGICAL ACTIVITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexadecanionic acid</td>
<td>Saturated fatty acid</td>
<td>Hypercholesterolemic, haemolytic, 5-alpha-reductase-inhibitor, antialopecic, antioxidant, nematicide.</td>
</tr>
<tr>
<td>Cis-9-Octadecanoic acid (Oleic acid)</td>
<td>Saturated fatty acid</td>
<td>Antioxidant, Anti-inflammatory, Hypercholesterolemic, haemolytic, 5-alpha-reductase-inhibitor, antialopecic, nematicide and Acidulent.</td>
</tr>
<tr>
<td>Dimethyl Phthalate</td>
<td>Aromatic dicarboxylic ester</td>
<td>Anticancer (Pharynx), Anthelmintic, Phagocytotic, Phosphodiesterase-Inhibitor.</td>
</tr>
<tr>
<td>Furan,2-hexyl</td>
<td>Furan</td>
<td>Antidote (Heavy metals), Hematonic, Hemoglobin Inducer.</td>
</tr>
<tr>
<td>(+)-Ascoctic acid,2,6-dihexadecanoate</td>
<td>Ascobates</td>
<td>Antioxidant, Imunebuster, wound healing agent.</td>
</tr>
<tr>
<td>3-Hydroxy-4-methoxy benzoic acid</td>
<td>Benzoic acid derivative</td>
<td>Acidulent, Arachidonic acid-Inhibitor, Urine acidifier.</td>
</tr>
<tr>
<td>4-Fluorobenzoic acid</td>
<td>Benzoic acid derivative</td>
<td>Acidulent, Arachidonic acid-Inhibitor, Urine acidifier, Antimicrobial agent.</td>
</tr>
</tbody>
</table>

Oleic acid is a monosaturated fatty acid, also called Omega-9 and like Omega-3 and Omega-6 fatty acids are less susceptible to spoilage than some other fats, therefore they can be used in foods as preservatives. It is also used in making creams, lotions, lipsticks due to its emollient activity. It is known to have cholesterolytic and cancer-preventing activities[11,12,13-14] n-Hexadecanic acid (Palmitic acid) and tetradecanoic acid (Myristic acid) are saturated fatty acids and are necessary for production of energy, hormones and for padding of organs. They are generally important in signaling processes and if not available may impair the function of growth factors in the cells and organs, [15-16] palmitic acid had also been proven to show leukemic causing apoptosis but not as in-vivo toxicity to tumour cells in mice. Palmitic acid is a lead compound of anti-cancer drugs. [17,18-20] Benzoic acid and Benzoic acid derivatives are prominent anti-infectives mainly used as preservatives in pharmaceutical industries. The presence of Benzoic acid, 4-Fluorobenzoic acid and 1-(+)-Ascoctic acid, 2,6-dihexadecanoate in the extracts of Z.tessmannii justifies their ethnomedicinal use as chewing sticks, for oral hygiene. Hence, this extracts could also be considered in the formulation of herbal oral lozenges.

CONCLUSION

The UV-VIS spectroscopy of root bark extract of Z.tessmannii identified simple phenol, alcohols and possibly polyphenols. Whereas peak at 335 nm could be attributed to carotenoids. FTIR spectroscopy, identified functional groups such as alkenes, alkanes, alcohols and esters while sixteen phyto-compounds were identified in the methanol and Ethyl acetate extracts of the plant by GC-MS analysis. The presence of these phyto-
com pounds in the plant provides a leading evidence for their use as chewing stick to keep the mouth fresh and odour free (antimicrobial effect). Hence, the root bark extracts could be used in the formulation of Herbal oral lozenges.

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Conflict of Interest: The authors report no conflicts of interest.

REFERENCES